



**Presentations at the 47th Interscience
Conference on Antimicrobial Agents and
Chemotherapy**

Chicago, Illinois, September 17-20th, 2007





Executive Summary

Monogram Virology and its collaborators presented 3 abstracts at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy in Chicago, Illinois. These abstracts focused on HIV tropism and included:

- **Co-receptor tropism predictions based on V3 loop sequence predictions in antiretroviral experienced patients.** This study indicated that if the sequence could be identified, the “state of the art” algorithms significantly underreported the presence of the X4 viruses
- **The prediction of disease progression by HIV co-receptor tropism in individuals with untreated chronic HIV infection.** The study revealed that patients with untreated chronic HIV infection with dual mixed tropic virus as compared to R5 tropic virus have a faster rate of HIV disease progression in all the measures used in the study
- **The enhancement of the Trofile™ assay to reliably detect minor CXCR4 using variants.** Results from this investigation showed that by modifying the Trofile™ assay the detection of X4 minor variants was enhanced 10 fold without altering the specificity for detection of X4 and R5 variants

Abstract Number: H-1028

Co-receptor Tropism Predictions Based on V3 Loop Sequence in Antiretroviral-Experienced Patients are Specific but Insensitive for the Detection of CXCR4-using Variants.

E. STAWISKI, J. WHITCOMB, E. COAKLEY, S. FRANSEN, E. PAXINOS, J. TOMA, T. WRIN; W. HUANG, C.J. PETROPOULOS;
Monogram Biosciences, South San Francisco, CA.

Background

Interest in co-receptor tropism (CRT) assays is growing with the availability of CCR5 antagonists for treatment of HIV-1 infection. The Trofile™ phenotypic assay is being used extensively to support the pre-clinical and clinical development of CRT inhibitors. The performance of genotype assays in heavily treatment-experienced patients – the likely target population for CCR5 antagonists – is unknown. Previous studies have highlighted technical difficulties in obtaining high quality envelope sequence and limitations of interpretation systems.

Methods

Samples from triple-class experienced patients were studied (N ~600). CRT (R5, X4, or dual/mixed) was determined by the Trofile™ assay. Population V3 loop sequences were determined using conventional techniques. CRT was predicted (R5 only or X4 using) according to published algorithms: modified 11/25 rule, position-specific scoring matrix, decision trees, or a support-vector machine model, trained on publicly available data.

Results

Based on Trofile™ results approximately half of the samples were defined as CXCR4-using; 25% had 6 or more mixtures in V3 (including variants with and without insertions or deletions) and were not interpretable. Another 25% had no mixtures in V3 at the protein level, and 50% had 1 to 5 mixtures in V3. Of the interpretable V3 sequences, overall concordance with phenotype ranged from 74 to 79%, and was characterized by high X4 specificity (90-100%) but low sensitivity (18-38%).

Conclusions

In treatment-experienced patients, who are likely candidates for treatment with CCR5 antagonists, envelope sequencing is hampered by diversity in variable loop length amongst subpopulations of viruses in approximately one quarter of patients. When a V3 sequence can be unambiguously determined, state of the art interpretation algorithms significantly under-report the presence of X4-using virus.

Comments on Abstract H-1028

NOTE: For the poster, further analysis was carried out. These updated results are shown with data in square brackets

- To assess the performance of co-receptor tropism prediction using the V3 loop sequence in treatment experienced individuals
- Approximately 600 triple class experienced patients were studied
- All patients had their viral tropism determined by Trofile™ and by V3 loop sequence prediction (using published algorithms including the modified 11/25 rule, position-specific scoring matrix, decision trees or a support-vector machine model, trained on publicly available data)
- Results:
 - By Trofile™ approximately 50% of these treatment experienced patients were defined as CXCR4 using
 - Twenty five percent [27%] had 6 or more mixtures in V3 and were uninterruptible
 - Twenty five percent had no V3 mixtures at the protein level
 - Fifty percent had 1 to 5 mixtures in V3
 - Of the interpretable results the concordance with phenotype ranged from 74-79% [73-85%] with high X4 specificity (90-100% [89-100%]) but low sensitivity (18-34% [22-48%])

Key points about Abstract H-1028

- In treatment experienced patients, envelope sequencing is hampered by diversity in variable loop length amongst subpopulations of viruses and was uninterruptible in 25-27 % of patients
- When a V3 sequence could be determined, the latest algorithms significantly underreported the presence of X4 viruses

Abstract Number: H-1027

Prediction of Disease Progression by HIV Co-Receptor Tropism (CRT) in persons (P) with Untreated Chronic HIV Infection

M. B. GOETZ¹, R. LEDUC², J. R. KOSTMAN³, A. LABRIOLA⁴, Y. LIE⁵, J. WEIDLER⁵, E. COAKLEY⁵, R. LUSKIN-HAWK⁶, The Terry Bein Community Programs For Clinical Research On Aids;

¹UCLA/VA Greater Los Angeles Hlth.care System, Los Angeles, CA, ²Univ. of Minnesota, Minneapolis, MN, ³UPHS/Presbyterian Med. Ctr., Philadelphia, PA, ⁴VA Med. Ctr., Washington, DC, ⁵Monogram BioSci., South San Francisco, CA, ⁶Saint Joseph Hosp., Chicago, IL.

Background: We assessed the effect of HIV CRT on the relative risk (RR) of progression to a composite outcome of CD4⁺ count <350, treatment initiation (Rx) or death in treatment-naïve P.

Methods: At entry all P were treatment-naïve and had ≥450 CD4⁺ cells/μL and a viral load (VL) of ≥1,000 HIV RNA copies/mL. CRT was assessed using the Trofile™ assay (Monogram).

Results: 32 P had dual/mixed R5/X4 (DM) CRT and 281 had R5 CRT. The median CD4⁺ count in P with R5 vs. DM CRT (635 vs. 571, p=0.10) and VL (4.05 vs 4.35 log₁₀ p=0.09) did not differ. P with R5 CRT were less often Latino/a (7.8% vs 25%, p=0.007), no other differences were found among demographic variables, HBV or HCV seropositivity rates, duration of known HIV infection (median 48 months) or study follow-up (median 52 months). P with DM CRT progressed more rapidly to the composite outcome of CD4⁺ count <350 (n=112), Rx (n=65) or death (n=8). The RR of progression to the composite outcome was 2.14 (p=0.002) for DM vs. R5 CRT, 2.05 per 1.0 log₁₀ higher VL (p<0.001) and 0.87 per 50 cell higher CD4⁺ count (p<0.001). The effect of DM CRT was also significant in separate analyses of time to CD4⁺ count <350 (RR=2.65, p <0.01) or time to Rx (RR=2.35, p<0.01). The results were unaffected by further adjustment for gender, age, race, MSM contact, HCV infection, known duration of HIV infection and prior AIDS diagnosis.

Conclusion: Untreated P with DM CRT vs. R5 CRT have a faster rate of HIV disease progression, whether assessed by a composite outcome of CD4⁺ count <350, Rx or death, or by separate analyses of time to CD4⁺ count <350 or Rx. Compared with R5 CRT, the effect on progression of having DM CRT was similar to a one log₁₀ increase in VL.

Comments on Abstract H-1027.

NOTE: For the poster, one additional patient was added to the analysis. Updated results are shown with data in square brackets.

- To assess the effect of HIV co-receptor tropism on disease progression in prospectively followed treatment naïve HIV-infected patients
- LTM cohort patients had to have ≥ 450 CD4⁺ cells/ μ L and a viral load (VL) of $\geq 1,000$ HIV RNA copies/mL to be eligible for this tropism analysis. Both RC and viral tropism were assessed using PhenoSense™ and Trofile™ (Monogram Biosciences)
- Baseline results
 - 32 patients had dual/mixed R5 / X4 (DM) tropic virus
 - 281 [282] patients had R5 virus
 - Median CD4⁺ counts were 635 cells/ μ L in patients with R5 tropic virus and 571 cells/ μ L in patients with DM tropic virus
 - Median viral loads were 4.05 [4.1] log₁₀ in patients with R5 tropic virus and 4.35 [4.4] log₁₀ in patients with DM tropic virus
- Results: Demographics
 - Patient's with R5 virus were less often Latino (7.8% vs. 25%, $p = 0.09$ [0.01]). No other differences were found
- Results: Progression
 - Patients with DM virus progressed more quickly in all composite outcomes (CD4⁺ <350, treatment initiation or death).
 - The relative risk of progression to the composite outcomes was 2.14 for DM vs. R5 virus ($p = 0.002$ [0.003]), 2.05 [2.02] per 1.0 log₁₀ higher VL ($p < 0.001$ [0.0001]) and 0.87 per 50 cell higher CD4⁺ count ($p < 0.001$ [0.0001])
 - The effect of DM tropism was also significant in separate analysis of time to CD4⁺ <350 (RR = 2.65 $p < 0.01$) or time to treatment initiation (RR = 2.35 [2.03] $p < 0.01$ [0.009])
 - These effects were observed in analyses that controlled for baseline factors such as CD4⁺ count, viral load, gender, age, race, MSM contact, HCV infection, known duration of HIV infection and prior AIDS diagnosis

Key points about Abstract H-1027.

- Untreated patients with DM tropic virus vs. R5 tropic virus have a faster rate of HIV disease progression in all measured composite outcomes
- Compared with R5 tropism, the impact of DM tropism was:
 - Similar to a 1.0 log₁₀ increase in VL
 - Greater than that of a 50 cell/ μ L decrease in CD4⁺ count

Abstract Number: H-1026

Enhancements to the Trofile HIV Coreceptor Tropism™ Assay Enable Reliable Detection of CXCR4-Using Subpopulations at Less Than 1%

J. D. REEVES¹, D. HAN², Y. LIU², T. WRIN², W. HUANG², E. COAKLEY², C. PETROPOULOS², N. PARKIN²;

¹Monogram Biosciences., South San Francisco, CA, ²Monogram Biosciences., South San Francisco, CA.

Background: Several coreceptor inhibitors (CIs) which block infection via CCR5 are in clinical development. The Trofile™ assay is useful for selection and monitoring of patients receiving CIs. In mixed envelope (*env*) populations, Trofile detects minor variants at 10% and 5% of the population with 100 and 83% sensitivity, respectively. Minor variants below the detection limit of Trofile can sometimes be identified by clonal analysis and may be selected following CI therapy. We are exploring approaches to increase minor CXCR4-using variant detection in the event that this will further optimize selection of patients who may benefit from CCR5 inhibitors.

Methods: R5, X4 and DM *env* clones and patient *env* populations were used to assess Trofile™ sensitivity following assay modifications to determine if minor CXCR4-using variants could be detected with enhanced sensitivity. A broad range of modifications were designed and evaluated to maximize the luciferase signal, including manipulation of virus input levels, (co)receptor levels, transfection and infection conditions.

Results: Env and tropism dependent differences in response to several modifications were observed. A combination of modifications designed to optimize detection of minority populations of CXCR4-using variants were identified. Using pre-defined mixtures of clones with R5 or X4 tropism, the sensitivity for detection of X4 minor variants was enhanced 10-fold, while preserving high specificity for detection of X4 and R5 variants.

Conclusions: We identified a series of assay modifications that individually and in combination enhanced detection of CXCR4-using envs in patient *env* pools. Specific combinations of modifications can be used to adjust assay sensitivity as required. Defining the level of sensitivity that is most clinically meaningful will entail retrospective evaluations of CCR5 inhibitor clinical trial samples with associated treatment outcome data.

Comments about Abstract H-1026

- The study aimed to increase the sensitivity of the Trofile™ assay in measuring minor CXCR4 using variants in patient derived HIV *env* populations. This would further optimize the selection of patients who may benefit from CCR5 co-receptor antagonists
- Methods:
 - R5, X4, DM and patient derived *env* populations were used to assess the sensitivity of the Trofile™ assay
 - Numerous modifications were made to the assay in an attempt enhance the sensitivity of the assay to measure minor CXCR4 using variants while maintaining its specificity for detection of X4 and R5 variants
 - Modifications included:
 - Maximization of the luciferase signal
 - Manipulation of the virus input levels
 - Manipulation of co-receptor levels
 - Manipulation of transfection and infection conditions
- Results:
 - A combination of modifications to optimize the detection of minor CXCR4 using variants were identified
 - Using a pre-defined mixture of clones with R5 and X4 tropism, the sensitivity for detection of X4 minor variants was enhanced 10 fold while preserving the high specificity for detection of X4 and R5 variants

Key points on Abstract H-1026

- By modifying the Trofile™ assay, the detection of X4 minor variants was enhanced 10 fold without sacrificing the specificity for detection of X4 and R5 variants
- Trofile™ assay sensitivity can be adjusted as required
- Defining the sensitivity of Trofile™ to that which is most clinically relevant will entail retrospective analysis of CCR5 co-receptor antagonists trials with their associated clinical outcome data